(19) Canadian Intellectual Property Office
An Agency of Industry Canada

Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

(11) **CA 2 412 181** (13) **A1**

(40) **09.12.2002**
(43) **09.12.2002**

(12)

(21) **2 412 181**

(22) **15.06.2001**

(51) Int. Cl. [7]: **C07K 5/06**, C07C 257/00, A61K 38/04

(85) **09.12.2002**

(86) **PCT/EP01/06814**

(87) **WO01/096366**

(30) **100 29 015.9 DE 15.06.2000**

(71)
CURACYTE AG,
Gollierstr. 70
80339, MUNCHEN, XX (DE).

(72)
SCHWEINITZ, ANDREA (DE).
STEINMETZER, TORSTEN (DE).
KUNZEL, SEBASTIAN (DE).
STURZEBECHER, JORG (DE).

(74)
GOUDREAU GAGE DUBUC

(54) INHIBITEURS DU FACTEUR DE COAGULATION XA

(54) DIPEPTIDE INHIBITORS FOR THE BLOOD-CLOTTING FACTOR XA

(57)

The invention relates to derivatives of amidino-benzylamine, especially derivatives of 4-amidino-benzylamine, with two bonded amino acids. These derivatives represent a novel group of highly active and very selective F Xa- inhibitors for treating cardiovascular diseases and thrombotic events.

Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2412181 A1 2002/12/09

(21) 2 412 181

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2001/06/15
(87) Date publication PCT/PCT Publication Date: 2002/12/09
(85) Entrée phase nationale/National Entry: 2002/12/09
(86) N° demande PCT/PCT Application No.: EP 2001/006814
(87) N° publication PCT/PCT Publication No.: 2001/096366
(30) Priorité/Priority: 2000/06/15 (100 29 015.9) DE

(51) Cl.Int.^{7}/Int.Cl.^{7} C07K 5/06, A61K 38/04, C07C 257/00

(71) Demandeur/Applicant:
CURACYTE AG, DE

(72) Inventeurs/Inventors:
STURZEBECHER, JORG, DE;
STEINMETZER, TORSTEN, DE;
KUNZEL, SEBASTIAN, DE;
SCHWEINITZ, ANDREA, DE

(74) Agent: GOUDREAU GAGE DUBUC

(54) Titre : INHIBITEURS DU FACTEUR DE COAGULATION XA
(54) Title: DIPEPTIDE INHIBITORS FOR THE BLOOD-CLOTTING FACTOR XA

(57) Abrégé/Abstract:
The invention relates to derivatives of amidino-benzylamine, especially derivatives of 4-amidino-benzylamine, with two bonded amino acids. These derivatives represent a novel group of highly active and very selective F Xa-inhibitors for treating cardiovascular diseases and thrombotic events.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
20. Dezember 2001 (20.12.2001)

PCT

(10) Internationale Veröffentlichungsnummer
WO 01/96366 A3

(51) Internationale Patentklassifikation[7]: C07K 5/06, A61K 38/04, C07C 257/00

(21) Internationales Aktenzeichen: PCT/EP01/06814

(22) Internationales Anmeldedatum:
15. Juni 2001 (15.06.2001)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
100 29 015.9 15. Juni 2000 (15.06.2000) DE

(71) Anmelder *(für alle Bestimmungsstaaten mit Ausnahme von US)*: **CURACYTE AG** [DE/DE]; Gollierstr. 70, 80339 München (DE).

(72) Erfinder; und

(75) Erfinder/Anmelder *(nur für US)*: **STÜRZEBECHER, Jörg** [DE/DE]; Hubertusstrasse 38, D-99094 Erfurt (DE). **STEINMETZER, Torsten** [DE/DE]; Ricarda-Huch-Weg 23, D-07743 Jena (DE). **KÜNZEL, Sebastian** [DE/DE]; St.-Jacob-Str. 6, D-07743 Jena (DE). **SCHWEINITZ, Andrea** [DE/DE]; Gustav-Fischer-Str. 15, D-07745 Jena (DE).

(74) Anwalt: **BÖSL, Raphael**; Bardehle, Pagenberg, Dost, Altenburg, Geissler, Isnbruck, Galileiplatz 1, 81679 München (DE).

(81) Bestimmungsstaaten *(national)*: AU, BR, CA, CR, JP, NO, NZ, US, ZA.

(84) Bestimmungsstaaten *(regional)*: europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Veröffentlicht:
— *mit internationalem Recherchenbericht*

(88) Veröffentlichungsdatum des internationalen Recherchenberichts: 25. April 2002

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

WO 01/96366 A3

(54) Title: DIPEPTIDE INHIBITORS FOR THE BLOOD-CLOTTING FACTOR XA

(54) Bezeichnung: DIPEPTIDISCHE HEMMSTOFFE FÜR DEN GERINNUNGSFAKTOR XA

(57) Abstract: The invention relates to derivatives of amidino-benzylamine, especially derivatives of 4-amidino-benzylamine, with two bonded amino acids. These derivatives represent a novel group of highly active and very selective F Xa-inhibitors for treating cardiovascular diseases and thrombotic events.

(57) Zusammenfassung: Die Erfindung betrifft Derivate des Amidino-Benzylamins, insbesondere solche des 4-Amidino-benzylamins, mit zwei gebundenen Aminosäuren, die eine neue Gruppe von hochaktiven und sehr selektiven F Xa-Hemmstoffen zur Behandlung kardiovaskulärer Erkrankungen und thrombotischer Ereignisse darstellen.

Inhibitors for the coagulation factor Xa

The invention relates to novel inhibitors for the coagulation factor Xa for the treatment of cardiovascular diseases and for the prevention of thromboembolic events.

The anticoagulants of the heparin type or the vitamin K antagonists presently employed clinically do not comply with all requirements for an "ideal" antithrombotic. Therefore alternatives are sought with low molecular weight inhibitors of the coagulation enzymes, especially of thrombin and factor Xa (F Xa). A particular advantage of F Xa inhibitors in comparison with thrombin inhibitors could be the lower tendency to bleeding which has been shown in various animal experiments. Thus the bleeding time was only minimally influenced in antithrombotically effective doses (J. M. Herbert et al., J. Pharmacol. Exp. Ther. 276, 1030-1038, 1996; K. Sato et al., Brit. J. Pharmacol. 123, 92-96, 1998).

The first nonpeptide compounds having a high affinity for F Xa were symmetrical bisbenzamidines (K_i = 13 nM for the most active compound BABCH) (J. Stürzebecher et al., Thromb. Res. 54, 245-252, 1998). The naphthamidine derivative DX-9065a has two basic groups and inhibits F Xa selectively with K_i = 24 nM (T. Hara et al., Thromb. Haemost. 71, 314-319, 1994). The inhibitor YM-60828 (K. Sato et al., Eur. J. Pharmacol. 339, 141-146, 1997), which is structurally related to DX-9065a, is even more active (K_i = 1.3 nM). In the meantime, a whole series of further bis-basic compounds have been described in which, for example, two benzamidine residues are linked via an oxazoline ring (K_i = 18 nM) (M.L. Quan et al., Bioorg. Med. Chem. Lett. 7, 2813-2818, 1997) or a carboxymethyl-alkyl chain (K_i = 34 nM) (T.P. Maduskuie

et al., J. Med. Chem. 41, 53-62, 1998). The disadvantage of the bis-basic compounds is, in particular, the low bioavailability after oral administration.

Inhibitors for F Xa which only contain one basic group have also been described. N-substituted amidinophenoxypyridines (K_i = 0.11 nM for BX-807834) were developed on the basis of BABCH (R. Mohan et al., Bioorg. Med. Chem. Lett. 8, 1877-1882, 1998; G.B. Phillips et al., J. Med. Chem. 41, 3557-3562, 1998). Amides of Nα-adamantyloxycarbonyl-3-amidinophenylalanine (K_i = 74 nM for the most active compound) are selective inhibitors of F Xa (S. Sperl et al., Biol. Chem. 381, 321-329, 2000), while Nα-arylsulfonyl-aminoacylated esters of 3-amidinophenylalanine have a low inhibitory action (K_i ≈ 840 nM for TAPAM) (J. Stürzebecher et al., Thromb. Res. 54, 245-252, 1998). WO 96/10022 discloses inhibitors which no longer have any strong charge at all (K_i = 3.0 nM for the most active compound).

Until now, only a few peptides which are derived from the substrate sequence Ile-Glu-Gly-Arg have been described as inhibitors of F Xa. The chloromethyl ketones described by Kettner and Shaw (Thromb. Res. 22, 645-652, 1981) inhibit F Xa irreversibly and are not suitable for in vivo applications. On the other hand, the peptides SEL 2489 (K_i = 25 nM) and SEL 2711 (K_i = 3 nM) are extremely active (J. A. Ostrem et al., Biochemistry 37, 1053-1059, 1998). Some peptidyl arginine aldehydes have also been described which, in addition to argininal in the P1 position, have a D-arginine or an unnatural basic amino acid in P3 (Z.H. Jonathan, Bioorg. Med. Lett. 9, 3459-3464, 1999). However, so far no peptidyl agmatine derivatives are known as inhibitors of F Xa, although this type of inhibitor has led to considerable advances in the further development of thrombin inhibitors. In this

case, the successes with compounds of the D-Phe-Pro-Arg type having a C-terminal agmatine and derivatives derived therefrom were particularly noteworthy. Picomolar K_i values were achieved for thrombin inhibition and the oral bioavailability was improved (T.J. Tucker et al., J. Med. Chem. 40, 1565-1569 and 3687-3693, 1997). In this case, however, no inhibition of F Xa was observed. For instance, melagatran, which has a 4-amidinobenzylamine residue at the C terminus and is very unspecific, inhibits F Xa with a K_i = 2.8 µM. On the other hand, trypsin (K_i = 4.0 nM) and thrombin (K_i = 2.0 nM) are inhibited more than three orders of magnitude more strongly (D. Gustafsson et al., Blood Coagul. Fibrinolysis 7, 69-79, 1996).

The invention is based on the object of specifying an active compound which is also suitable for therapeutic applications and inhibits the coagulation factor Xa with high activity and specificity and which can be prepared with the lowest possible synthesis expenditure.

Surprisingly, it has been found that acylated amidinobenzylamine according to the general formula I shown in Patent claim 1, in particular compounds of 4-amidinobenzylamine in which X, R_1, R_2 and R_3 result in natural and/or unnatural amino acids, inactivate factor Xa very efficaciously and selectively and effectively inhibit the coagulation of human blood plasma. Amidinobenzylamine in this case forms a particularly active inhibitor of factor Xa if the amidino group is in the 4-position, glycine and D-serine tert-butyl ether are bonded as amino acids and if the compound has an N-terminal protective group R_4 composed of an aryl- or aralkylsulfonyl residue.

Besides factor Xa, other enzymes were markedly less inhibited by the glycine derivatives, such that the derivatives of amidinobenzylamine according to the

invention are a novel group of highly active and very selective F Xa inhibitors. In contrast to this, compounds which carry no H as R_1 (e.g. alanine derivatives) no longer selectively inhibit factor Xa, but are also strong inhibitors of trypsin, thrombin and plasmin.

The compounds are as a rule present as salts with mineral acids, preferably as hydrochlorides, or as salts with suitable organic acids.

The compounds of the general formula I can be prepared in a manner known in principle, as described below:

The starting compound 4-cyanobenzylamine is prepared from 4-cyanobenzyl bromide via Gabriel synthesis (G. Wagner and I. Wunderlich, Pharmazie 32, 76-77, 1977; B.C. Bookser and T.C. Bruice, J. Am. Chem. Soc. 113, 4208-4218, 1991). The Boc-protected acetyloxamidinobenzylamine is obtained from the 4-cyanobenzylamine thus prepared. The coupling of the further amino acids and of the protective group R_4 is carried out by means of standard coupling methods using Boc as an N-terminal protective group. The second amino acid can also be coupled directly as an N-aryl- or N-aralkylsulfonyl-protected amino acid. The peptide analogs are synthesized sequentially, beginning from the acetyloxamidinobenzylamine. Most of the products crystallize well and can thus be simply purified. The purification of the inhibitors is carried out in the last stage by means of preparative, reversed-phase HPLC.

The invention will be illustrated in greater detail below with the aid of three working examples:

Working example 1:

Synthesis of benzylsulfonyl-D-Ser(Bz)-Gly-4-amidino-benzylamide x HCl

1.1 Boc-4-cyanobenzylamide

20 g (0.151 mol) of 4-cyanobenzylamine were dissolved in 300 ml of H_2O, 150 ml of dioxane and 150 ml of 1 N NaOH. 37.5 ml of di-tert-butyl dicarbonate were added dropwise with ice cooling and the mixture was stirred at 0°C for one hour and at room temperature for a further 24 hrs. The dioxane was removed i.v. and the aqueous residue was extracted 3 times with ethyl acetate. The combined extracts were washed 3 times with 5% strength $KHSO_4$ solution and 3 times with saturated NaCl solution, dried over Na_2SO_4 and concentrated i.v. (white crystals). HPLC: acetonitrile/H_2O, elution in 44.1% acetonitrile; yield: 30.48 g (0.131 mol), 87%.

1.2 Boc-4-acetyloxamidinobenzylamide

According to Judkins et al. (Synthetic Comm. 26, 4351-4367, 1996), 30.48 g (0.131 mol) of Boc-4-cyanobenzylamide were dissolved in 300 ml of abs. ethanol with 13.65 g (0.197 mol) of hydroxylamine x HCl and 34 ml (0.197 mol) of DIEA. The solution was refluxed for 2 hrs and stirred at room temperature overnight. The batch was then concentrated i.v., the residue was dissolved in about 200 ml of acetic acid and the solution was treated with 18.67 ml (0.197 mol) of acetic anhydride. After 1 hr, it was again concentrated, dissolved in ethyl acetate and washed 3 times each with 5% strength $KHSO_4$ solution and saturated NaCl solution at 0°C. After drying over Na_2SO_4 and concentrating i.v., a white powder precipitated. HPLC: acetonitrile/H_2O, elution in 32.0% acetonitrile; yield: 31.3 g (0.102 mol) 78%.

1.3 4-Acetyloxamidinobenzylamine x HCl

5 mmol of Boc-4-acetyloxamidinobenzylamide are dissolved in 20 ml of 1 N HCl in glacial acetic acid and the solution is allowed to stand at room temperature for 45 min. It is then largely concentrated i.v., and the product is precipitated using dry diethyl ether, filtered off on a frit, washed again with fresh ether and dried i.v. On account of the quantitative reaction, the product was employed for the next synthesis step without further purification.

1.4 Boc-Gly-4-acetyloxamidinobenzylamide

The coupling of Boc-Gly-OH (Orpegen, Heidelberg) to 4-acetyloxamidinobenzylamine was carried out according to Frérot et al. (Tetrahedron 47, 259 ff., 1991). To this end, 2.064 g (9.3 mmol) of 4-acetyloxamidinobenzylamine x HCl and 1.629 g (9.3 mmol) of Boc-Gly-OH were dissolved in about 25 ml of DMF. 4.84 g (9.3 mmol) of PyBOP and 3.878 ml (27.9 mmol) of TEA were then added at 0°C and the pH was adjusted to 9 using TEA. After stirring at room temperature for 1 hr, the mixture was concentrated i.v., taken up in ethyl acetate and subjected to acidic, basic and neutral washing 3 times each, dried and concentrated. Yield: 3 g (8.2 mmol) 88%.

1.5 Boc-Gly-4-amidinobenzylamide x AcOH

3 g (8.2 mmol) of Boc-Gly-4-acetyloxamidinobenzylamide were dissolved in 200 ml of 90% strength acetic acid. 300 mg of 10% palladium on activated carbon were then added under argon. Argon was replaced by a hydrogen atmosphere and the batch was hydrogenated for 24 hrs with vigorous stirring. The catalyst was filtered off and the filtrate was concentrated i.v.. Yield: 2.9 g (7.9 mmol) 96%.

1.6 H-Gly-4-amidinobenzylamide x 2 HCl

2.9 g (7.9 mmol) of Boc-Gly-4-amidinobenzylamide were dissolved in 100 ml of 1 N HCl in glacial acetic acid and the solution was allowed to stand at room temperature for 45 min. It was then largely concentrated i.v. and precipitated using dry diethyl ether, then filtered off on a frit and the product was again washed with fresh ether. After drying the product i.v., it was used without further purification for the synthesis according to item 1.8.

1.7 Benzylsulfonyl-D-Ser(Bz)-OH

229 mg (1.173 mmol) of H-D-Ser(Bz)-OH and 408 µl (2.345 mmol) of DIEA were dissolved in 50 ml of 50% acetonitrile. 335 mg (1.76 mmol) of benzylsulfonyl chloride were then added and the mixture was stirred at room temperature for 12 hrs. It was concentrated i.v., taken up using ethyl acetate and subjected to acidic and neutral washing 3 times each. After drying over sodium sulfate, it was concentrated i.v. Yield: 289 mg (0.827 mmol) 71%.

1.8 Benzylsulfonyl-D-Ser(Bz)-Gly-4-amidinobenzylamide x TFA

151 mg (0.433 mmol) of benzylsulfonyl-D-Ser(Bz)-OH and 121 mg (0.433 mmol) of H-Gly-4-amidinobenzylamide x 2 HCl were dissolved in a little abs. DMF. 225 mg (0.433 mmol) of PyBOP and 230 µl (1.32 mmol) of DIEA were added with ice cooling. After stirring at room temperature for 1 hr, the mixture was concentrated i.v. and the product was purified by means of HPLC (acetonitrile/H_2O, 0.1% trifluoroacetic acid, elution in 37.4% acetonitrile). Yield: 232 mg (0.356 mmol) 82%.

Working example 2:

Inhibition of F Xa by selected compounds having Y = amidino

R_4	R_3 configuration	R_3	R_2	X-R_1	Amidino position	K_b µM
H	L	CH_2-OH	H	CH_2	4	> 1000
Boc	L	CH_2-OH	H	CH_2	4	110
H	D	CH_2-OH	H	CH_2	4	> 1000
Ac	D	CH_2-OH	H	CH_2	4	> 1000
Bz-SO_2	D	CH_2-OH	H	CH_2	4	3.0
Bz-SO_2	D	CH_2-O-Bz	H	CH_2	4	0.050
Bz-SO_2	D	CH_2-O-tBu	H	CH_2	4	0.030
Bz-SO_2	D	CH_2-O-tBu	H	CH_2-CH_3	4	0.044
H	D	CH_2-O-Bz	H	CH_2	3	140
Boc	D	CH_2-O-Bz	H	CH_2	3	93
Bz-SO_2	D	CH_2-O-Bz	H	CH_2	3	84

Determination of the inhibitory action

For the determination of the inhibitory action, 200 µl of tris buffer (0.05 M, 0.154 M NaCl, 5% ethanol, pH 8.0; contains the inhibitor), 25 µl of substrate (Moc-D-Nle-Gly-Arg-pNA in H_2O; Pentapharm Ltd., Basle, Switzerland) and 50 µl of F Xa (from Rind, Diagnostic Reagents Ltd, Thame, GB) were incubated at 25°C. After 3 min, the reaction was interrupted by addition of 25 µl of acetic acid (50%) and the absorption at 405 nm was determined by means of microplate reader (MR 5000, Dynatech, Denkendorf, Germany). The K_i values were

determined according to Dixon (Biochem. J. 55, 170-171, 1953) by linear regression by means of a computer program. The K_i values are the mean of at least three determinations.

Working example 3:

Inhibition of the coagulation of human plasma by benzylsulfonyl-D-Ser(Bz)-Gly-4-amidinobenzylamide

Concentration µM	Prolongation of the coagulation time (%)	
	aPTT	PT
3.3	385	386
1.7	260	266
0.83	185	198
0.42	146	153
0.21	122	127
0.1	111	119

Determination of the inhibition of coagulation

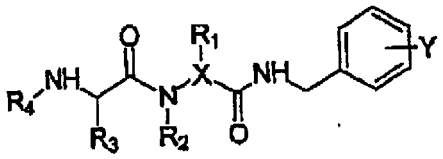
For the determination of the prothrombin time (PT), 100 µl of thromboplastin (Dade, Unterschleißheim) and 100 µl of inhibitor, dissolved in $CaCl_2$ (0.05 M, 5% ethanol) were incubated at 37°C for 2 min and the coagulation was started by addition of 100 µl of human citrate plasma. For the determination of the activated partial thromboplastin time (aPTT), 100 µl of human citrate plasma were incubated with 100 µl of aPTT reagent (Roche Diagnostics, Mannheim) at 37°C for 3 min and the coagulation was started by addition of 100 µl of inhibitor, dissolved in $CaCl_2$ (0.05 M, 5% ethanol). The coagulation times were determined using the Thrombotrack coagulometer (Immuno, Heidelberg).

Abbreviations used:

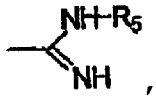
Ac	acetyl
Boc	tert-butyloxycarbonyl
Bz	benzyl
DIEA	diisopropylethylamine
DMF	N,N-dimethylformamide
i.v.	in vacuo
PyBOP	benzotriazol-1-yl-N-oxytris-(pyrrolidino)phosphonium hexafluorophosphate
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
tBu	tert-butyl

Patent claims

1. Inhibitors for the coagulation factor Xa of the general formula I:



in which the substituent Y occurs in the 3- or 4-position and is an amidino group



in which R_5 has an H, an OH or a carbonyl residue -CO-R or oxycarbonyl residue -COO-R, where R can be a branched or unbranched alkyl having 1-16 C atoms, a substituted or unsubstituted aryl or heteroaryl residue or a substituted or unsubstituted aralkyl or heteroaralkyl residue,

X is a CH group or N,

R_1 is H or a branched or unbranched alkyl having 1-8 C atoms or $(CH_2)_n$-OH where n = 1-5,

R_2 is an H or a branched or unbranched alkyl having 1-8 C atoms,

R_3 is a branched or unbranched alkyl having 1-8 C atoms or a $(CH_2)_n$-O-R_6, $(CH_2)_n$-S-R_6 or $(CH_2)_n$-NH-R_6 where n = 1-5 and R_6 is a branched or unbranched alkyl having 1-16 C atoms, a substituted or unsubstituted aryl or heteroaryl residue or a substituted or an unsubstituted aralkyl or heteroaralkyl residue and

R_4 is a sulfonyl residue $-SO_2-R$, a carbonyl residue -CO-R, an oxycarbonyl residue -COO-R or an H, where R is a branched or unbranched alkyl having 1-16 C atoms, a substituted or an unsubstituted aryl or heteroaryl residue, a substituted or unsubstituted aralkyl or heteroaralkyl residue, an adamantyl residue, a camphor residue or a cyclohexylmethyl residue.

2. Inhibitors according to Claim 1, characterized in that, in the amidinobenzylamide, the amidino group is in the 4-position and that the amino acids Gly and D-Ser(tBu) and, as R_4, an aryl or an aralkylsulfonyl residue are bonded thereto.

3. Use of the inhibitors according to Claim 1 for the preparation of orally, subcutaneously, intravenously or transdermally administrable medicaments for the prevention or treatment of thromboembolic diseases.

4. Use according to Claim 3, characterized in that the inhibitors are employed as medicaments in the form of tablets, coated tablets, capsules, pellets, suppositories, solutions or patches, etc.

5. Use of the inhibitors according to Claim 1 as a diagnostic agent, in particular in thrombotic events.